

CLAIMS

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1. A method for purifying a calcium ion-binding protein from a sample containing said protein using a cation exchange carrier, wherein said method comprises contacting
5 the sample with the cation exchange carrier in the presence of calcium ions to let the protein be adsorbed to the exchange carrier; and eluting the adsorbed calcium ion-binding protein from the exchange carrier by decreasing or removing a concentration of calcium ions and/or adding
10 counter ions other than calcium ions.

2. The method of claim 1 wherein the adsorption step is performed in the presence of 5 to 100 mM calcium ions.

3. The method of claim 2 wherein the adsorption
15 step is performed in the presence of 10 to 30 mM calcium ions.

4. The method of any one of claims 1 to 3 wherein the adsorption step is performed at a flow rate of 1 to 150
cm/h.

20 5. The method of claim 4 wherein the adsorption step is performed at a flow rate of 15 to 100 cm/h.

6. The method of claim 4 wherein the adsorption step is performed at a flow rate of 50 to 80 cm/h.

7. The method of claim 1 wherein the elution step
25 is performed by decreasing a concentration of calcium ions

to less than 5 mM.

8. The method of claim 1 wherein the elution step is performed by adding 1 to 500 mM of counter ions other than calcium ions.

5 9. The method of claim 1 wherein the elution step is performed by adding 50 to 500 mM of counter ions other than calcium ions.

10 10. The method of claim 1 wherein the elution step is performed by adding 50 to 300 mM of counter ions other than calcium ions.

11. The method of claim 1, 8, 9 or 10 wherein said counter ions are selected from the group consisting of Na⁺, Li⁺ and K⁺.

Sub A2 15 12. The method of claim 1, 7, 8, 9, 10 or 11 wherein the elution step is performed at a flow rate of 1 to 150 cm/h.

13. The method of claim 12 wherein the elution step is performed at a flow rate of 30 to 100 cm/h.

20 14. The method of claim 12 wherein the elution step is performed at a flow rate of 30 to 80 cm/h.

15. The method of claim 1 wherein the cation exchange carrier is selected from the group consisting of SP-Sepharose, CM-Sepharose, CM-cellulose, SE-cellulose, S-Spherodex and SP-Spherosil.

Sub A3 25 16. The method of any one of claims 1 to 15

wherein the calcium ion-binding protein is selected from the group consisting of Annexins I, II, III, IV, V, VI and VII.

17. The method of any one of claims 1 to 16 wherein the sample contains a calcium ion-binding protein prepared by the genetic recombination technique.

18. The method of any one of claims 1 to 17 wherein the adsorption and elution steps are performed at pH 5 to 10.

19. The method of claim 18 wherein the adsorption and elution steps are performed at pH 8 to 9.5.

20. The method of claim 19 wherein the adsorption and elution steps are performed at pH 9.

21. The method of claim 1 wherein the adsorption step is performed in the presence of 10 to 30 mM calcium ions at pH 8 to 9.5 at a flow rate of 15 to 100 cm/h; the elution step is performed at a flow rate of 30 to 80 cm/h by decreasing a concentration of calcium ions to less than 5 mM or by adding 50 to 300 mM counter ions selected from the group consisting of Na⁺, Li⁺ and K⁺; the cation exchange carrier is SP-Sepharose; the calcium ion-binding protein is Annexin V; the sample contains Annexin V prepared by the genetic recombination technique; and protease is removed from the sample.

22. The method of claim 1 wherein the adsorption step is performed in the presence of 10 to 30 mM calcium

ions at pH 8 to 9.5 at a flow rate of 15 to 100 cm/h; the elution step is performed at a flow rate of 30 to 80 cm/h by decreasing a concentration of calcium ions to less than 5 mM or by adding 500 mM counter ions selected from the group consisting of Na⁺, Li⁺ and K⁺; the cation exchange carrier is SP-Sepharose; the calcium ion-binding protein is Annexin VI; the sample contains naturally occurring Annexin VI; and protease is removed from the sample.

23. A method for purifying a calcium ion-binding protein from a sample containing said protein using a cation exchange carrier, wherein said method comprises contacting the sample with the cation exchange carrier in the presence of calcium ions to let the protein be adsorbed to the carrier.

24. The method of claim 23 wherein said method is performed in the presence of 5 to 100 mM calcium ions.

25. The method of claim 24 wherein said method is performed in the presence of 10 to 30 mM calcium ions.

26. The method of any one of claims 23 to 25 wherein said method is performed at a flow rate of 1 to 150 cm/h.

27. The method of claim 26 wherein said method is performed at a flow rate of 15 to 100 cm/h.

28. The method of claim 26 wherein said method is performed at a flow rate of 50 to 80 cm/h.

29. The method of claim 23 wherein the cation exchange carrier is selected from the group consisting of SP-Sepharose, CM-Sepharose, CM-cellulose, SE-cellulose, S-Spherox and SP-Spherosil.

5 *Sub 04* 30. The method of any one of claims 23 to 29 wherein the calcium ion-binding protein is selected from the group consisting of Annexins I, II, III, IV, V, VI and VII.

31. The method of any one of claims 23 to 30 wherein the sample contains a calcium ion-binding protein
10 prepared by the genetic recombination technique.

32. The method of any one of claims 23 to 31 wherein the method is performed at pH 5 to 10.

33. The method of claim 32 wherein the method is performed at pH 8 to 9.5.

15 34. The method of claim 33 wherein the method is performed at pH 9.

Sub 05 35. A calcium ion-binding protein of high purity in a single peak as determined by gel filtration chromatographic analysis, obtained by the method of any one
20 of claims 1 to 34.